The Journal of Immunology

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J Immunol 2007; 178:5473-5479; ;
doi: 10.4049/jimmunol.178.9.5473
http://www.jimmunol.org/content/178/9/5473

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Down-Regulation of IL-7Rα Expression in Human T Cells via DNA Methylation[1]

Hang-Rae Kim, Kyung-A Hwang, Ki-Chan Kim, and Insoo Kang[2]

IL-7 is critical for the development and survival of T cells. Recently, we found two subsets of human CD8$^+$ T cells expressing IL-7Rα^{high} and IL-7Rα^{low} with different cell survival responses to IL-7. Although these CD8$^+$ T cell subsets have differential *IL-7Rα* gene expression, the mechanism for this is unknown. DNA methylation is an important gene regulatory mechanism and is associated with the inactivation of gene expression. Thus, we investigated a role for DNA methylation in differentially regulating *IL-7Rα* gene expression in human CD8$^+$ T cells and Jurkat T cells. IL-7Rα^{high}CD8$^+$ T cells had decreased methylation in the *IL-7Rα* gene promoter compared with IL-7Rα^{low}CD8$^+$ T cells and Jurkat T cells with low levels of IL-7Rα. Treating Jurkat T cells with 5-aza-2′-deoxycytidine, which reduced DNA methylation, increased IL-7Rα expression. Plus, the unmethylated *IL-7Rα* gene promoter construct had higher levels of promoter activity than the methylated one as measured by a luciferase reporter assay. These findings suggest that DNA methylation is involved in regulating IL-7Rα expression in T cells via affecting *IL-7Rα* gene promoter activity, and that the methylation of this gene promoter could be a potential target for modifying IL-7-mediated T cell development and survival. ***The Journal of Immunology,*** **2007, 178: 5473–5479.**

Interleukin 7, a member of the common cytokine receptor γ-chain family of cytokines, is critically involved in the development and maintenance of naive and memory CD8$^+$ and CD4$^+$ T cells (1–6). IL-7 is produced largely by epithelial cells in the thymus and bone marrow (5) and promotes CD8$^+$ T cell survival by up-regulating Bcl-2, an antiapoptotic molecule, via sequential activation of JAK1, JAK3, and STAT5 (7, 8). The IL-7R complex that consists of two chains, the high-affinity IL-7Rα chain and common cytokine receptor γ-chain (9), dictates cell survival responses to IL-7. For example, in mice infected with lymphocyte choriomeningitis virus, CD8$^+$ T cells expressing IL-7Rα^{high} had better survival and differentiation into memory cells compared with CD8$^+$ T cells expressing IL-7Rα^{low} (3). Similarly, when CD8$^+$ T cells from IL-7R intact and knockout mice were adoptively transferred to wild-type mice, cells from IL-7R knockout mice had decreased survival compared with those from IL-7R-intact mice (1).

Recently, we identified two subsets of cells expressing IL-7Rα^{high} and IL-7Rα^{low} in human peripheral CD8$^+$ T cells (10). IL-7Rα^{low}CD8$^+$ T cells had decreased cell signaling and survival responses to IL-7 compared with IL-7Rα^{high}CD8$^+$ T cells, which demonstrates the physiologic significance of differential IL-7Rα expression on CD8$^+$ T cell subsets (10). The underlying molecular mechanism for the generation and maintenance of IL-7Rα^{low} CD8$^+$ T cells is largely unknown. However, IL-7Rα^{low}CD8$^+$ T cells had decreased mRNA expression of the *IL-7Rα* gene compared with IL-7Rα^{high}CD8$^+$ T cells, which indicates that the regulation of *IL-7Rα* gene expression operates differently in IL-7Rα^{high} and IL-7Rα^{low} CD8$^+$ T cells in homeostasis (10).

The regulation of gene expression is a complicated process that is achieved through the action of selective transcriptional factors, as well as via epigenetic regulatory mechanisms, including DNA methylation and histone modifications (11). In mammals, DNA methylation occurs at cytosines within CpG dinucleotides and is regulated by DNA methyltransferases (Dnmts)[3] that add methyl groups to cytosines (12). DNA hypomethylation is generally associated with active gene expression (12), and differential methylation of DNA has been noticed in T cells at different stages of cell differentiation (13–15). For example, hypomethylation of the *IFN-γ* gene occurred during in vitro differentiation of cells into Th1 cells that produced IFN-γ in humans (16). Furthermore, hypomethylation of the same gene was observed in memory T cells that produced high levels of IFN-γ, but not in naive T cells, which produced only low levels of IFN-γ in mice infected with lymphocyte choriomeningitis virus (17).

In the current study, we investigated the role for DNA methylation in differentially regulating IL-7Rα expression in human CD8$^+$ T cells in homeostasis. The results of our study demonstrate that IL-7Rα^{high}CD8$^+$ T cells, including naive (CD45RA$^+$CCR7$^+$) and IL-7Rα^{high} memory (CD45RA$^{+/-}$CCR7$^-$) CD8$^+$ T cells, have decreased methylation in the *IL-7Rα* gene promoter compared with IL-7Rα^{low} memory CD8$^+$ T cells and Jurkat T cells expressing low levels of IL-7Rα. The treatment of Jurkat T cells with 5-aza-2′-deoxycytidine (5-aza-dC), a Dnmt inhibitor that reduces DNA methylation, increased the mRNA and protein expression of IL-7Rα. In addition, a luciferase reporter assay showed that the unmethylated form of the *IL-7Rα* gene promoter construct had higher levels of promoter activity than the methylated form of the

Department of Internal Medicine, Section of Rheumatology, Yale University School of Medicine, New Haven, CT 06520

Received for publication December 7, 2006. Accepted for publication February 16, 2007.

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[1] This work was supported in part by grants from the National Institutes of Health (K08 AR49444-02), the American College of Rheumatology, the Arthritis Foundation, the American Foundation for Aging Research, and Claude D. Pepper Older Americans Independence Center at Yale University School of Medicine (P30AG21342 NIH/NIA).

[2] Address correspondence and reprint requests to Dr. Insoo Kang, Department of Internal Medicine, Section of Rheumatology TAC S541C, Yale School of Medicine, P.O. Box 208031, New Haven, CT 06520. E-mail address: Insoo.kang@yale.edu

[3] Abbreviations used in this paper: Dnmt, DNA methyltransferase; 5-aza-dC, 5-aza-2′-deoxycytidine; EM, effector memory; GABPα, guanine- and adenine-binding protein α; GFI-1, growth factor independence 1.

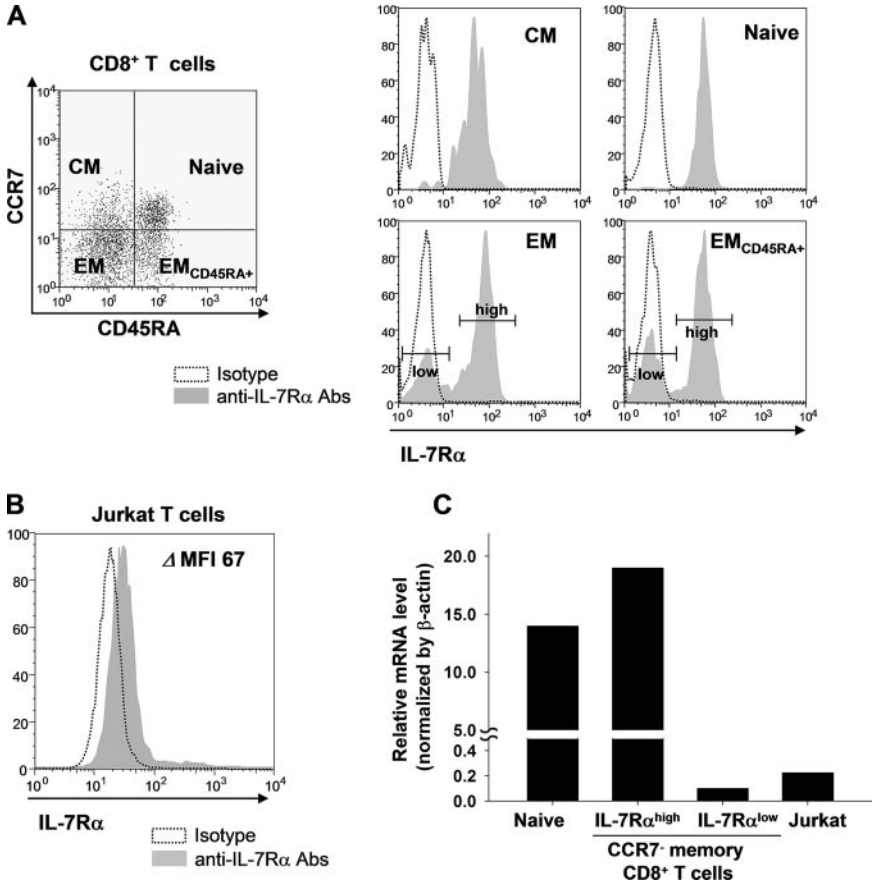

FIGURE 1. IL-7Rα expression by CD8$^+$ T cell subsets and Jurkat T cells. IL-7Rα expression was measured on naive (CD45RA$^+$CCR7$^+$), CM (CD45RA$^-$CCR7$^+$), EM (CD45RA$^-$CCR7$^-$), and CD45RA$^+$ EM (EM$_{CD45RA+}$, CD45RA$^+$ CCR7$^-$) CD8$^+$ T cell subsets in healthy subjects as well as on Jurkat T cells. *A* and *B*, Representative histograms of IL-7Rα (shaded) and isotype control (open) staining from >10 independent experiments. *C*, *IL-7Rα* mRNA expression as measured by real-time PCR. PBMCs from a healthy adult were stained with Abs to CD8, CD45RA, CCR7, and IL-7Rα and sorted into naive, CCR7$^-$ memory IL-7Rα^{high}, and IL-7Rα^{low} CD8$^+$ T cells. The expression of the *IL-7Rα* gene in freshly sorted naive, IL-7Rα^{high} CCR7$^-$ memory, IL-7Rα^{low}CCR7$^-$ memory CD8$^+$ T cells, and Jurkat T cells was measured using real-time PCR. Data were normalized to *β-actin* gene expression in individual samples. Results are representative data from four independent experiments.

IL-7Rα gene promoter construct, further supporting the role for DNA methylation in regulating *IL-7Rα* gene expression. These findings suggest that a novel mechanism is involved in differentially regulating IL-7Rα expression by T cells through altering DNA methylation of the *IL-7Rα* gene promoter, and that methylation of this gene promoter could be a potential target for modifying IL-7-mediated T cell development and survival.

Materials and Methods

Cells and FACS sorting

This work was approved by the institutional review committee of Yale University. Human peripheral blood was drawn from healthy adult subjects after obtaining informed consent. As previously described (10), PBMCs were purified and stained with Abs to CD8, CD45RA, CCR7, and IL-7Rα (all from BD Pharmingen, except anti-IL-7Rα Abs from R&D Systems). Stained cells were sorted into naive (CD45RA$^+$CCR7$^+$), IL-7Rα^{high}, and IL-7Rα^{low}CCR7$^-$ memory (CD45RA$^{+/-}$CCR7$^-$) CD8$^+$ T cells using a FACSAria (BD Immunocytometry Systems). Jurkat T cells (E6-1 clone, TIB-153) were obtained from the American Type Culture Collection.

Cell culture, flow cytometry, and real-time PCR

Sorted CD8$^+$ T cell subsets and Jurkat T cells were cultured in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. In experiments blocking Dnmts, 5-aza-dC (Sigma-Aldrich) at final concentrations of 0.025–0.4 μM or PBS was added to Jurkat T cells (18). In stimulating CD8$^+$ T cells, cells were incubated for 4 or 14 days in a 48-well tissue culture plate coated with anti-CD3 Abs (BD Pharmingen) at 10 μg/ml or PBS in the presence of anti-CD28 Abs (10 μg/ml; BD Pharmingen). Some cells were additionally stimulated with IL-4 (50 ng/ml), IL-7 (20 ng/ml), IL-15 (50 ng/ml), and IFN-α (2×10^3 U/ml; R&D Systems) (19). In measuring the expression of IL-7Rα, PBMCs were stained with goat anti-human IL-7Rα or isotype Abs as well as with Abs to CD8, CD45RA, CCR7, and donkey anti-goat IgG. Jurkat T cells were stained with goat anti-human IL-7Rα or isotype Abs, followed by donkey anti-goat IgG. Stained cells were analyzed on a FACSCalibur (BD Immunocytometry Systems). Flow cytometry data were analyzed using FlowJo software (Tree Star). Total RNA was isolated from sorted CD8$^+$ T cell subsets and Jurkat T cells and used for cDNA synthesis. The real-time PCR for *IL-7Rα* gene expression were performed as previously described (10). All results were normalized to *β-actin* gene expression.

Determining DNA methylation using Pyrosequencing

Genomic DNA was isolated from Jurkat T cells and sorted CD8$^+$ T cell subsets using a DNeasy Tissue Kit (Qiagen). Extracted DNA was amplified using high-fidelity DNA polymerase, and the sequence of the *IL-7Rα* gene promoter (−1346 to −1 bp) was verified by sequencing (GenBank accession number DQ821273) (20, 21). To determine the methylation status of CpG sequences in the *IL-7Rα* gene promoter, bisulfite modification and Pyrosequencing were performed at EpigenDX as previously described (22). Bisulfite modification, which converted unmethylated cytosines to uracils, was performed using an EZ DNA Methylation Kit (Zymo Research) according to the manufacturer's instructions. The efficiency of bisulfite treatment, measured by the conversion of cytosines not contained in CpG sites into uracils, was >97% in all experiments. The promoter sequence of the *IL-7Rα* gene was PCR amplified by using 100 ng bisulfite-modified DNA and 200 μM each of dNTP, 1× PCR buffer, 1.5 mM $MgCl_2$, and 0.2 μM PCR primers (for site −1064: forward, 5′-TGATATATAAATGGGTGAGGTTGT-3′, reverse, 5′-biotin-CTTTTTTTTTCCCAAATAAACCTT-3′; for sites −656, −586, −563, and −555: forward, 5′-GTGAAATTTGGAAGTTGGAGGTAA-3′, reverse, 5′-biotin-CCCAAATTCAAACAATTCTCCT-3′; and for site −435: forward, 5′-TTGGGAGGTGAAAATTGTAGTGAG-3′, reverse, 5′-biotin-TAAATATTCCCTACAACCCCCACA-3′). The PCR condition was as follows: 15 min at 94°C, 45 cycles of 15 s at 95°C, 30 s at 58°C, and 15 s at 72°C, followed by a final extension step for 5 min at 72°C. The biotinylated PCR product was purified and made single-stranded to act as a template in a Pyrosequencing reaction. Four sequencing primers (for site −1064: 5′-TGAGGTTGTATTTTAAATGA-3′; for site −656, −586, −563, and −555: 5′-TAGATTTTTTTAAAGTGGGT-3′, 5′-AGGTAGATTATTTGAGGTTA-3′; and for site −435: 5′-GGAGGTGAAAATTGTAGTG-3′) were designed to determine the CpG dinucleotide methylation status. The Pyrosequencing

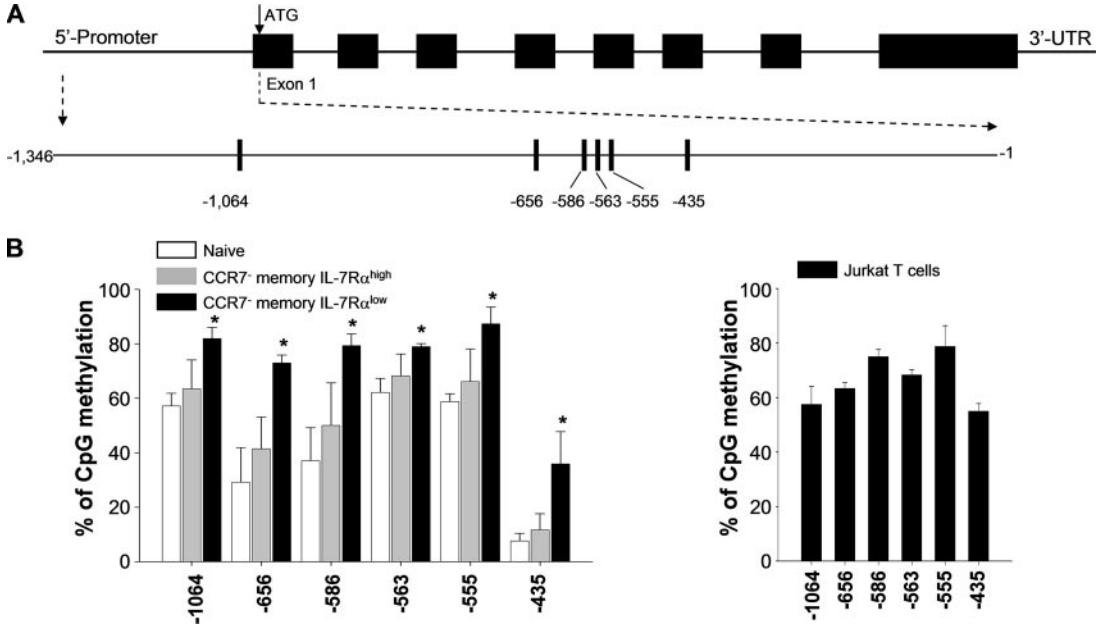

FIGURE 2. The methylation status of CpG sequences in the *IL-7Rα* gene promoter in CD8$^+$ T cell subsets and Jurkat T cells. *A*, The genomic region of human *IL-7Rα* gene encompassing eight exons is schematically shown. ■ and | with numbers, exons and the locations of CpG dinucleotides relative to the start codon in the *IL-7Rα* gene promoter (−1346 to −1), respectively. *B*, Methylation status of six individual CpG dinucleotides in the *IL-7Rα* gene promoter. PBMCs from healthy adult subjects were sorted into naive (n = 3), IL-7Rα^{high} (n = 5), and IL-7Rα^{low} (n = 5) CCR7$^-$ memory (CD45RA$^{+/-}$ CCR7$^-$) CD8$^+$ T cell subsets. Genomic DNA was isolated from sorted CD8$^+$ T cell subsets and Jurkat T cells (n = 3), treated with bisulfite, amplified by PCR, and sequenced as described in *Materials and Methods*. Each bar indicates the mean and SD for individual CpG sites (x-axis) from the different CD8$^+$ T cell subsets. ∗, Differences in DNA methylation between IL-7Rα^{low} and naive cells as well as between IL-7Rα^{low} and IL-7Rα^{high} cells for individual CpG sites were statistically significant ($p < 0.05$ for all sites by the Student t test).

reactions were performed on a PSQ HS96 Pyrosequencing system (Biotage) and data were analyzed using its methylation-analysis software.

Reporter gene construction, methylation, and reporter gene transfection assay

The *IL-7Rα* gene promoter region was amplified using high-fidelity DNA polymerase (Invitrogen Life Technologies) with primers containing *Kpn*I and *Xho*I restriction sites. The *IL-7Rα* gene promoter construct (pGL-IL7RαP) was produced by cloning a 1346-bp fragment of the *IL-7Rα* gene promoter fragment into the *Kpn*I/*Xho*I sites of pGL3-basic vector (Promega), and verified by sequencing. In methylating the *IL-7Rα* gene promoter construct, pGL-IL7RαP construct and pGL3-basic vector were incubated for 1 h at 37°C in the presence or absence (control, mock) of *Sss*I methylase (New England Biolabs) with 80 μM *S*-adenosylmethionine (23, 24). The extent of methylation status was confirmed by digestion with *Hpy*CH4IV, a methylation-sensitive restriction enzyme. Methylated and unmethylated constructs were ethanol-precipitated after phenol-chloroform extraction and were transfected into Jurkat T cells suspended in Nucleofector Solution V (Amaxa) using an Amaxa nucleofector apparatus (Amaxa) according to the manufacturer's instructions. The pRL-TK (Promega) was cotransfected as an internal control for transfection efficiency. Transfected cells were harvested after 24 h and used for measuring luciferase and *Renilla* activities according to the manufacturer's instructions (Promega).

Results

IL-7Rα gene expression is different in IL-7Rα^{high} and IL-7Rα^{low} CD8$^+$ T cells and Jurkat T cells with IL-7Rα^{low} expression

Two different subsets of CD8$^+$ T cells expressing IL-7Rα^{high} and IL-7Rα^{low} were identified in human peripheral blood as previously reported (10). Naive (CD45RA$^+$CCR7$^+$) and central memory (CD45RA$^-$CCR7$^+$) CD8$^+$ T cells were homogeneously IL-7Rα^{high} cells (Fig. 1*A*). In contrast, CCR7$^-$ memory CD8$^+$ T cells, including effector memory (EM; CD45RA$^-$CCR7$^-$) and CD45RA$^+$ EM (EM$_{CD45RA+}$, CD45RA$^+$CCR7$^-$) CD8$^+$ T cells had IL-7Rα^{high} and IL-7Rα^{low} cells (Fig. 1*A*). We also measured the expression of IL-7Rα on Jurkat T cells, a human leukemic cell line. Of interest, these leukemic T cells also had low expression of IL-7Rα similar to IL-7Rα^{low}CD8$^+$ T cells (Fig. 1*B*). We next measured the expression levels of *IL-7Rα* mRNA in naive and IL-7Rα^{high} CCR7$^-$ memory CD8$^+$ T cells as well as in IL-7Rα^{low} CCR7$^-$ memory CD8$^+$ T cells and Jurkat T cells. The expression of *IL-7Rα* mRNA was lower in IL-7Rα^{low}CCR7$^-$ memory CD8$^+$ T cells and Jurkat T cells compared with naive and IL-7Rα^{high} CCR7$^-$ memory CD8$^+$ T cells (Fig. 1*C*).

DNA methylation in the IL-7Rα *gene promoter is lower in naive and IL-7Rα^{high} memory CD8$^+$ T cells than in IL-7Rα^{low} memory CD8$^+$ T cells and Jurkat T cells*

The gene regulatory mechanism is a complicated process that involves discriminatory activation of transcriptional factors as well as epigenetic modifications of DNA and histones (11). A growing body of evidence indicates that methylation of cytosines within CpG dinucleotides in genes is an important mechanism for differentially regulating transcriptions of specific genes in mammalian cells, including T cells (12–15). Thus, we have investigated whether methylation of the *IL-7Rα* gene promoter has a role in differentially regulating the expression of *IL-7Rα* mRNA in human primary CD8$^+$ T cells and Jurkat T cells. First, we identified six sites of CpG dinucleotides, potential sites of DNA methylation, within the human *IL-7Rα* gene promoter (−1346 to −1 bp relative to the start codon) based on the gene sequence (20, 21). These sites are located at −1064, −656, −586, −563, −555, and −435 bp relative to the start codon ATG (Fig. 2*A*). Second, we measured the methylation status of these six CpG sites in naive, IL-7Rα^{high}, and IL-7Rα^{low}CCR7$^-$ memory CD8$^+$ T cells. In addition, the methylation status of the same CpG sites in Jurkat T cells was analyzed.

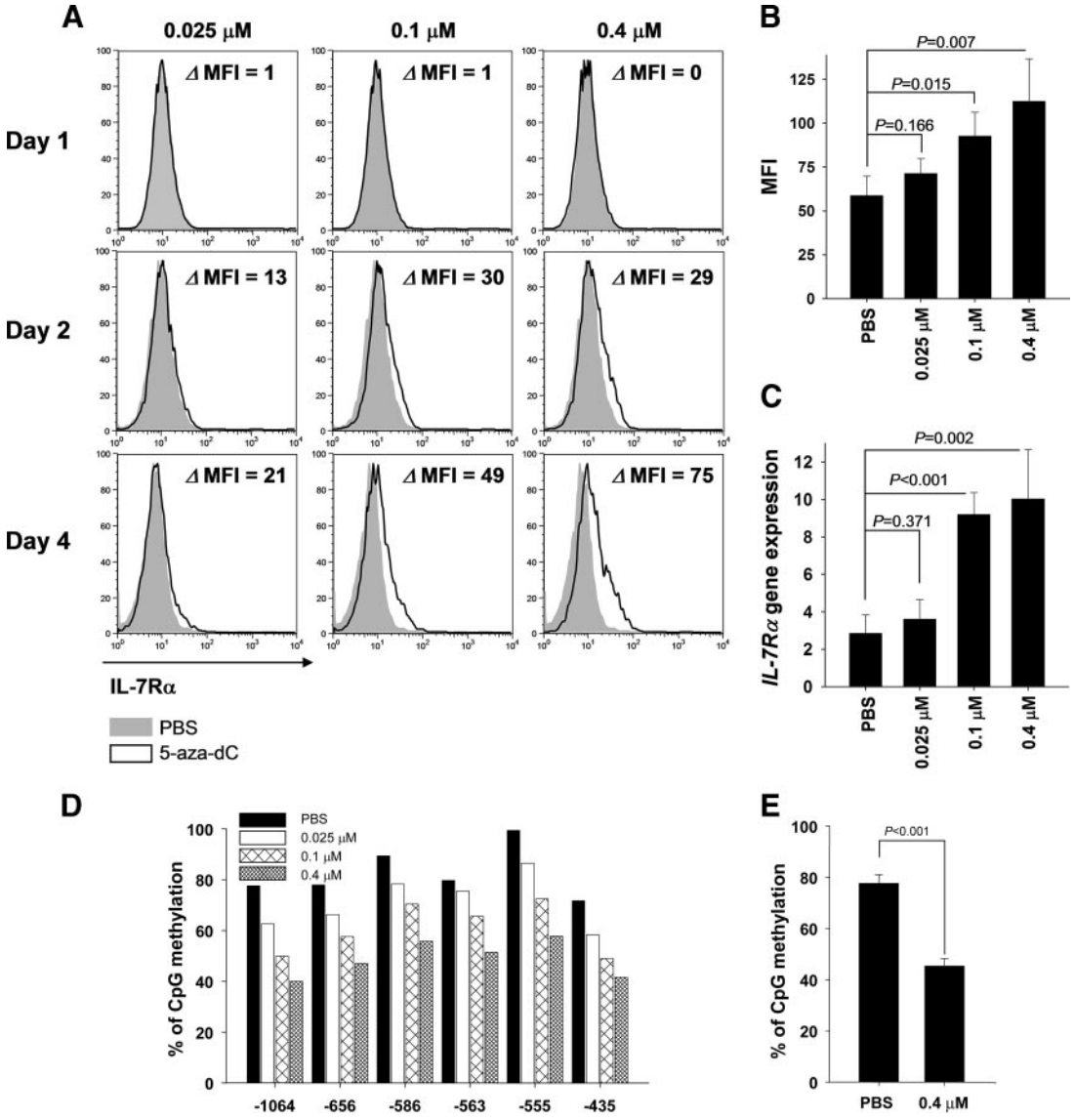

FIGURE 3. 5-aza-dC up-regulates the expression of IL-7Rα by Jurkat T cells. Jurkat T cells were cultured for 1, 2, and 4 days with 5-aza-dC (0.025, 0.1, or 0.4 μM) or PBS. *A*, Cells were stained with anti-IL-7Rα Abs or isotype Abs and analyzed on a flow cytometer. Numbers on histograms indicate the differences in the mean fluorescent intensity of IL-7Rα staining (ΔMFI) between samples treated with PBS and 5-aza-dC. Results are representative data from six independent experiments. *B* and *C*, Comparing mean fluorescence intensity of IL-7Rα staining (*B*) and mRNA expression (*C*) of the *IL-7Rα* gene among Jurkat T cells treated with PBS and different doses of 5-aza-dC (four samples for each treatment group). The expression of *IL-7Rα* mRNA was measured by real-time PCR. Graphs (*C*) show relative expression of *IL-7Rα* mRNA. Data were normalized to *β-actin* gene expression in individual samples. *D*, The methylation status of six CpG dinucleotides in the *IL-7Rα* gene promoter was determined in PBS- or 5-aza-dC-treated Jurkat T cells at day 4 of tissue culture that were analyzed in *A*. *E*, Comparing DNA methylation in the *IL-7Rα* gene promoter between Jurkat T cells treated with PBS and 5-aza-dC (0.4 μM; four samples for each treatment group). A value of *p* was obtained by the Student *t* test.

In all subsets of $CD8^+$ T cells and Jurkat T cells, methylation was concentrated at the five upstream CpG sites (positions −1064, −656, −586, −563, and −555; Fig. 2*B*). The least methylated site was at −435, regardless of IL-7Rα expression by T cells. In comparing the methylation status of the six CpG sites in the *IL-7Rα* gene promoter among the $CD8^+$ T cell subsets and Jurkat T cells, the methylation was lower in cells expressing IL-7Rα^{high}, including naive and IL-7R$\alpha^{high}CCR7^-$ memory $CD8^+$ T cells compared with cells expressing IL-7Rα^{low}, including IL-7Rα^{low} $CCR7^-$ memory $CD8^+$ T cells and Jurkat T cells (Fig. 2*B*). The differences between IL-7Rα^{low} and naive cells, as well as between IL-7Rα^{low} and IL-7Rα^{high} cells, for individual CpG sites were all statistically significant ($p < 0.05$ for all sites by the Student *t* test).

5-aza-dC up-regulates the expression of IL-7Rα by Jurkat T cells via inhibiting DNA methylation

5-aza-dC is a nucleoside analog of cytidine that specifically inhibits DNA methylation by trapping Dnmts during cell replication (25, 26). This chemical can affect gene expression by inducing hypomethylation of DNA (17, 26). Thus, we studied whether the treatment of cells expressing IL-7Rα^{low} with 5-aza-dC could up-regulate the expression of IL-7Rα by reducing methylation of the *IL-7Rα* gene promoter. In this experiment, Jurkat T cells were used because primary IL-7Rα^{low} memory $CD8^+$ T cells poorly proliferate in response to TCR triggering (10), and the effect of 5-aza-dC requires cell replication (25, 26). Treating Jurkat T cells with 5-aza-dC up-regulated the expression of IL-7Rα mRNA and

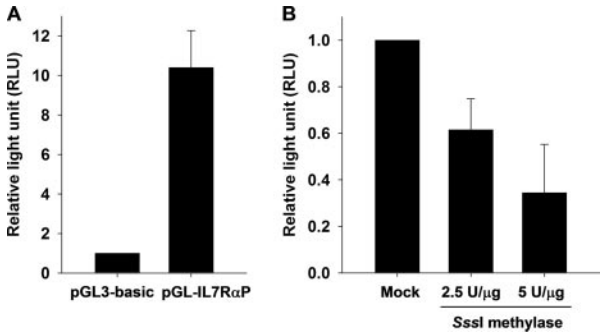

FIGURE 4. *IL-7Rα* gene promoter activity is affected by DNA methylation. *A*, The pGL-IL7RαP was constructed by inserting the promoter region of the human *IL-7Rα* gene (−1346 to −1 bp) into the pGL3-basic vector. The pGL-IL7RαP and pGL3-basic vector were transfected into Jurkat T cells, with pRL-TK vector as an internal control. Promoter activity is expressed as relative light units (RLU) to the pGL3-basic vector. The results are expressed as the mean ± SD from four samples for each group. *B*, The pGL-IL7RαP was methylated using *Sss*I methylase (0, 2.5, and 5 U/μg; see details in *Materials and Methods*). Methylated and unmethylated (mock) pGL-IL7RαP constructs were transfected into Jurkat T cells. The data were normalized to the unmethylated constructs (mock), of which activity was set as 1. The results are expressed as the mean ± SD from four samples for each group.

protein in a dose-dependent manner (Fig. 3, *A–C*). Jurkat T cells treated with 0.4 μM 5-aza-dC had the highest up-regulation of IL-7Rα compared with cells treated with 0.025 and 0.1 μM 5-aza-dC. In analyzing DNA methylation, Jurkat T cells treated with 5-aza-dC had decreased DNA methylation in the *IL-7Rα* gene promoter at days 2 and 4 of tissue culture compared with cells treated with PBS (Fig. 3, *D* and *E*, day 2; data not shown).

Methylated and unmethylated forms of the IL-7Rα *gene promoter have different promoter activities*

To determine the promoter activity of methylated and unmethylated forms of the *IL-7Rα* gene promoter, we constructed a pGL-IL7RαP plasmid containing a human *IL-7Rα* gene promoter region (1346 bp) from human $CD8^+$ T cells. The promoter activity of the *IL-7Rα* gene promoter fragment was demonstrated by measuring luciferase activity relative to pGL3-basic vector alone. The luciferase activity of the pGL-IL7RαP construct was 10-fold higher than the pGL3-basic vector (Fig. 4*A*). To measure the effect of DNA methylation in the *IL-7Rα* gene promoter, pGL-IL7RαP and pGL3-basic vector were methylated and transfected into Jurkat T cells. The methylated form of the *IL-7Rα* gene promoter construct

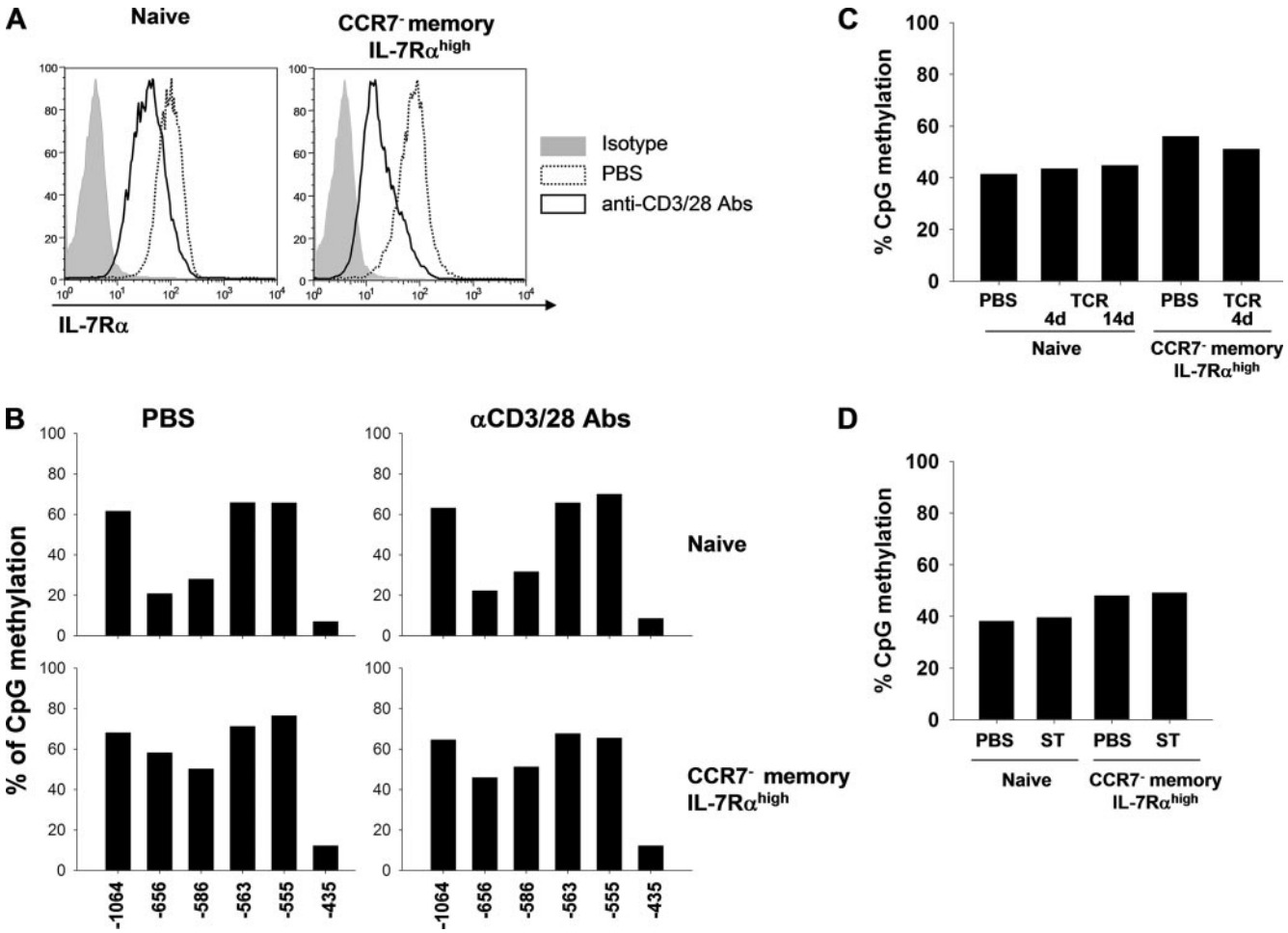

FIGURE 5. TCR triggering and cytokines suppresses IL-7Rα expression, but not the methylation status of the *IL-7Rα* gene promoter in $IL\text{-}7R\alpha^{high}CD8^+$ T cells. PBMCs were sorted as described in Fig. 2. *A–C*, Sorted cells were cultured for 4 or 14 days with anti-CD3 Abs at 10 μg/ml or PBS in the presence of anti-CD28 Abs (10 μg/ml). *A*, At day 4, cells were stained with anti-IL-7Rα or isotype Abs and analyzed with flow cytometry. *B*, At day 4, genomic DNA isolated from cells was analyzed for the methylation status of six individual CpG dinucleotides in the *IL-7Rα* gene promoter. *C*, The average methylation of all six CpG dinucleotides in the *IL-7Rα* gene promoter at days 4 and 14. *D*, Some sorted cells were cultured for 4 days with anti-CD3 Abs at 10 μg/ml in the presence of anti-CD28 Abs (10 μg/ml), IL-4 (50 ng/ml), IL-7 (20 ng/ml), IL-15 (50 ng/ml), and IFN-α (2×10^3 U/ml; labeled as ST) or PBS. Results are representative data from three independent experiments.

had lower levels of promoter activities compared with the unmethylated form (Fig. 4*B*). This finding further supports the role for DNA methylation in the regulation of *IL-7Rα* gene expression.

TCR triggering and cytokines induce the down-regulation of IL-7Rα expression by naive and IL-7Rα^{high} memory CD8$^+$ T cells without altering DNA methylation of the IL-7Rα *gene promoter*

TCR triggering is known to rapidly suppress IL-7Rα expression by T cells (10, 27). Thus, we determined whether TCR triggering-mediated suppression of IL-7Rα expression was related to an alteration in DNA methylation of the *IL-7Rα* gene promoter. Naive and IL-7Rα^{high}CCR7$^-$ memory CD8$^+$ T cells were sorted and incubated for 4 or 14 days in the presence of anti-CD3 and -CD28 Abs or PBS. The suppressed expression of IL-7Rα protein and mRNA by CD8$^+$ T cells was confirmed by flow cytometry (Fig. 5*A*) and real-time PCR (data not shown). Despite the decreased IL-7Rα expression by IL-7Rα^{high}CD8$^+$ T cells, including naive and memory CD8$^+$ T cells in response to TCR stimulation, the methylation patterns of the *IL-7Rα* gene promoter in these cells remained unchanged (Fig. 5, *B* and *C*). Furthermore, we stimulated IL-7Rα^{high}CD8$^+$ T cells with a combination of anti-CD3/-CD28 Abs, as well as IFN-α, IL-4, IL-7, and IL-15, which also are known to suppress IL-7Rα expression on T cells (19) (IFN-α; H. R. Kim and I. Kang, unpublished observation). However, there was no change noticed in DNA methylation in the *IL-7Rα* gene promoter in such stimulated cells, while these cells still had decreased IL-7Rα expression (Fig. 5*D*). These findings suggest that the mechanism(s) involved in generating IL-7Rα^{low} memory CD8$^+$ T cells with increased levels of DNA methylation in the *IL-7Rα* gene promoter in vivo may not be simply dependent on the factors that are associated with suppressing IL-7Rα expression in vitro, and that there are several different mechanisms in regulating *IL-7Rα* gene expression in T cells that operate differently depending on cell activation stages.

Discussion

IL-7 is critically involved in the development and survival of CD8$^+$ T cells in humans and mice (1–6). Despite the presence of CD8$^+$ T cells expressing IL-7Rα^{high} and IL-7Rα^{low} CD8$^+$ T cells with different survival responses to IL-7, it is largely unknown how these cells differentially regulate the expression of IL-7Rα. The current study addressed this issue, focusing on DNA methylation that is an important regulatory mechanism for gene expression. We found that naive and IL-7Rα^{high} memory CD8$^+$ T cells had lower levels of DNA methylation in the *IL-7Rα* gene promoter than in IL-7Rα^{low} memory CD8$^+$ T cells, suggesting the potential role for DNA methylation in differentially regulating *IL-7Rα* gene expression by human CD8$^+$ T cells. In fact, this finding is in line with the results of other studies reporting an inverse correlation of DNA methylation with active gene expression (13–15). Of interest, Jurkat T cells were found to have low expression of IL-7Rα mRNA and protein (Fig. 1, *B* and *C*), which were similar to those of IL-7Rα^{low} primary CD8$^+$ T cells. The methylation patterns of the *IL-7Rα* gene promoter were also similar between these cells (Fig. 2*B*). These findings suggest that the methylation patterns of the *IL-7Rα* gene promoter are even preserved in T cells that have undergone neoplastic transformation and proliferation. This observation is consistent with the principle of the epigenetic mechanisms by which cells inherit the characteristics of gene expression that determine cellular functions from ancestor cells (11). The difference in methylation of the *IL-7Rα* gene promoter between IL-7Rα^{high} and IL-7Rα^{low} CD8$^+$ T cells likely has physiologic significance in the survival of CD8$^+$ T cells. Indeed, we recently found that IL-7Rα^{high}CD8$^+$ T cells, including naive and IL-7Rα^{high} memory cells, had better signaling and survival responses to IL-7 compared with IL-7Rα^{low} memory CD8$^+$ T cells (10), indicating that the level of IL-7Rα expression by CD8$^+$ T cells directly dictate their survival in response to IL-7.

In our study, the methylation status of IL-7Rα^{high}CCR7$^-$ memory CD8$^+$ T cells was similar to that of naive CD8$^+$ T cells that have different cell phenotypes and functions compared with CCR7$^-$ memory CD8$^+$ T cells (Fig. 2*B*). This finding suggests that the differential regulation of *IL-7Rα* gene expression by DNA methylation occurs independently of other cell functions and differential markers, such as CCR7 and CD45RA. Although it is still unknown how altered methylation in the *IL-7Rα* gene promoter affects the transcription of this gene, the decreased *IL-7Rα* gene expression could be the result of direct blocking of the binding of transcriptional factors that have CpG sites in their recognition motifs (12). Alternatively, DNA methylation could indirectly alter gene expression by interacting with methyl-CpG-binding proteins as well as histone deacetylases and histone methyltransferases that are involved in modifications of chromatic structure (11).

The role for DNA methylation in regulating *IL-7Rα* gene expression is further supported by the results of two experiments in our study. First, Jurkat T cells treated with Dnmt inhibitor 5-aza-dC had increased mRNA and protein expression of IL-7Rα with reduced DNA methylation in the *IL-7Rα* gene promoter (Fig. 3). The change in DNA methylation occurred dose dependently and was found at all of the CpG sites of the *IL-7Rα* gene promoter (Fig. 3, *D* and *E*). Second, the unmethylated form of the *IL-7Rα* gene promoter construct had higher levels of promoter activity than the methylated form of the same promoter when they were transfected into Jurkat T cells (Fig. 4*B*). These findings demonstrate the role for DNA methylation in differentially regulating the expression of IL-7Rα by primary CD8$^+$ T cells and Jurkat T cells. The results of our study also raise the possibility of altering IL-7Rα expression in T cells by targeting DNA methylation of the *IL-7Rα* gene promoter which can affect the survival of memory CD8$^+$ T cells in infection, autoimmunity, and malignancy. In fact, azacytidine, an inhibitor of DNA methylation, has been clinically used to treat myelodysplastic syndrome, including chronic myelomonocytic leukemia (28).

TCR stimulation is known to down-regulate IL-7Rα expression on T cells (10, 27). Thus, we measured DNA methylation in the *IL-7Rα* gene promoter in IL-7Rα^{high}CD8$^+$ T cells, including naive and IL-7Rα^{high} memory cells, after stimulating them with anti-CD3/-CD28 Abs up to 14 days. Of interest, stimulated IL-7Rα^{high} cells had no change in DNA methylation in the *IL-7Rα* gene promoter, despite the down-regulation of IL-7Rα protein and mRNA expression (Fig. 5, *A* and *C*; mRNA data now shown). In addition, the combination of anti-CD3/-CD28 Abs and other cytokines including IFN-α, IL-4, IL-7, and IL-15 that can suppress IL-7Rα expression on T cells (19) (IFN-α; H. R. Kim and I. Kang, unpublished observation) did not change DNA methylation of the *IL-7Rα* gene promoter (Fig. 5*D*). These findings suggest several points. First, an additional factor(s) appears to be necessary for changing the DNA methylation of the *IL-7Rα* gene promoter in IL-7Rα^{high}CD8$^+$ T cells. Second, we suspect that such findings are secondary to the existence of several different mechanisms in regulating *IL-7Rα* gene expression in T cells, and that these mechanisms operate differently depending on cell activation stages. Indeed, it has been reported that the transcriptional factors, guanine- and adenine-binding protein α (GABPα) and growth factor independence 1 (GFI-1), play a role in regulating *IL-7Rα* gene expression by T cells. GABPα was required for the expression of the *IL-7Rα* gene by mouse and human T cells

(29), whereas GFI-1 was involved in down-regulating *IL-7Rα* gene expression on mouse lymph node T cells (19). Previously, we found that the levels of *GABPα* and *IL-7Rα* mRNA were down-regulated in human peripheral IL-7Rα^{high}CD8$^+$ T cells in response to TCR stimulation, supporting the role for GABPα in differentially regulating *IL-7Rα* expression (10). However, in the same study, there was no inverse correlation noticed between *IL-7Rα* and *GFI-1* mRNA expression (10), which raises the question on the role for this transcriptional factor in regulating *IL-7Rα* gene expression in human peripheral CD8$^+$ T cells. Our observations suggest that DNA methylation appears to be a dominant mechanism in regulating *IL-7Rα* gene expression in resting IL-7Rα^{high} and IL-7Rα^{low} T cells, whereas transcriptional factor(s) such as GABPα has a leading role in controlling the same gene expression in activated T cells via TCR triggering.

In summary, our study shows that DNA methylation in the *IL-7Rα* gene promoter is different among Jurkat T cells and CD8$^+$ T cell subsets with different levels of IL-7Rα expression and that DNA methylation in the *IL-7Rα* gene promoter affects its activity. Furthermore, we have demonstrated that reducing methylation of the *IL-7Rα* gene promoter can up-regulate IL-7Rα mRNA and protein expression by Jurkat T cells. These findings suggest that a novel mechanism is involved in differentially regulating IL-7Rα expression in T cells through altering the DNA methylation of the *IL-7Rα* gene promoter and that methylation of this gene promoter could be a potential target for modifying IL-7-mediated T cell development and survival. The results of our study are unique by providing a new research and clinical venue in the IL-7-mediated regulation of T cell survival via epigenetics.

Acknowledgments

We thank Drs. Susan Kaech and Alexia Belperron for critical reviews of this manuscript.

Disclosures

The authors have no financial conflict of interest.

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